Epidemiology of Antimicrobial Drug Resistance in *Salmonella, Escherichia coli* And Other Selected Pathogens of Livestock

Summary

This report briefly summarizes the epidemiology of antimicrobial drug resistance in animals in different countries, as presented at the WHO conference on "The Medical Impact of the Use of Antimicrobials in Food Animals" in October 1997 (World Health Organization, 1998). Approximately 31 papers were presented by investigators from Australia, Belgium, Canada, China, Denmark, France, Germany, Russia, Scotland, Sweden, Switzerland, United Kingdom, and the United States. This description of the epidemiology of antimicrobial drug resistance in livestock is focused primarily on two important zoonotic pathogens, *Salmonella spp.* and *Escherichia coli*. In addition, the epidemiology of antimicrobial drug resistance in livestock was discussed briefly for the following pathogens: *Staphylococcus aureus, Serpulina hyodysenteriae*, *Campylobacter*, *Salmonella* Typhimurium DT104, and respiratory pathogens. Finally, resistance to several antimicrobials including vancomycin, streptogramins, and quinolones, is discussed.

Widespread resistance to old and to recently developed antimicrobial drugs is occurring in several pathogens that are commonly associated with diseases in animals and humans. In general, long-term trends in the prevalences of antimicrobial drug resistance were not available from many countries because many surveillance systems were organized only recently. Where available, data suggest that the prevalence of resistance of some infectious agents to some antimicrobial drugs used in livestock is increasing, and it is high already in some situations.

Definitions and Terminology:

multidrug resistant isolate -- a bacterial isolate that is resistant to more than one antimicrobial drug.
monodrug resistant isolate -- a bacterial isolate that is resistant to one antimicrobial drug.
R-type -- resistance type.
MIC -- minimum inhibitory concentration. MIC refers to the lowest concentration of an antimicrobial drug that will inhibit the growth of a bacterium *in vitro*.
prevalence of resistance-- the number of isolates of a specific bacterium that are resistant to a specific antimicrobial drug divided by the number of isolates that were tested (expressed as a percentage)
quinolones -- antimicrobial drugs (e.g. nalidixic acid) that are targets for DNA gyrase, an essential bacterial enzyme that is responsible for introducing superhelical twists into bacterial DNA.
fluoroquinolones -- antimicrobial drugs (e.g. enrofloxacin) that are derivatives of quinolones and are used in animals and humans. Fluoroquinolones are characterized by a fluorine atom at position 6 and an amine group at position 7.
glycopeptides -- a family of antimicrobial drugs.
group therapy -- a therapeutic regimen in which an antimicrobial drug is given to numerous animals simultaneously (e.g., via feed or water).
individual therapy -- a therapeutic regimen in which an antimicrobial drug is given to each animal separately (e.g., via injection).

Antimicrobial Resistance of Salmonella

Resistance of *Salmonella spp.* in the U.S.

The 1,041 specimens collected by the U.S.'s National Antimicrobial Resistance Monitoring System (NARMS) were from cattle (49.7%), swine (32.9%), chickens (12.0%), and turkeys (5.9%). Non-clinical isolates were acquired from the USDA's National Dairy Heifer Evaluaiton Project, the 1994 Cattle on Feed Evaluation, and the Food Safety Inspection Service during 1994-1995, and from an on-farm swine epidemiologic survey. The clinical isolates were selected randomly from the reference database of the National Veterinary Services Laboratory. Of these 1,041 baseline animal isolates that were tested for resistance, 59.6% were resistant to no (i.e., were susceptible to all) antibiotics, 11.8 % were resistant to one antibiotic, and 13.5% were resistant to two antibiotics (Tollefson et al., 1998).

The prevalences of resistance among *Salmonella* isolates were 34% for tetracycline, 28% for sulfamethoxazole, 13% for ticarcillin and ampicillin, 8% for neomycin, and 7% for piperacillin. The six resistant serotypes that were represented were *S.* Derby, *S.* Typhimurium variety Copenhagen, *S*. Typhimurium, *S.* Agona, *S.* Cholerasuis, and *S*. Hadar.

Resistance of *Salmonella spp.* in France

During the years 1994 and 1995, 15,878 *Salmonella* isolates were tested for resistance, of which 3,962 isolates were from animals (Brisabois et al., 1997). Of the 3,962 isolates from animals, 1,181 (29.8%) were from bovine and 2,438 (61.5%) were from poultry. Among the multiresistant or monoresistant *Salmonella*, eight resistant serotypes were found. These serotypes were *S*. Typhimurium, *S*. Enteritidis, *S*. Virchow, *S*. Newport, *S*. Hadar, *S*. Saintpaul, *S*. Montevideo, *S*. Infantis, and *S*. Regent. The prevalence of resistance was 63.7% for the bovine isolates and 33.6% for the poultry isolates. The prevalence of resistance in the 1,790 isolates from the environment was 34.2% (n = 613). A high prevalence of resistance to ampicillin, streptomycin, chloramphenicol and tetracycline was found (Brisabois et al., 1997). Serotype *S.* Typhimurium, the most prevalent antibiotic resistant serotype among the bovine isolates, represented 94.0% of all bovine *Salmonella* isolates that were resistant. The prevalence of resistance among all *S.* Typhimurium was 91.0%. There have been two observed changes in antimicrobial resistance since monitoring of *Salmonella* began in France around 1997: (1) a decrease in monoresistant isolates, and (2) an increase in multiresitant isolates towards four to five antibiotics, including a major phenotype referred to as R-type ACSSuT (ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline).

Resistance of *Salmonella spp.* in Sweden

Salmonella Typhimurium and *S*. Dublin are isolated from production animals only sporadically in Sweden. Thus, Sweden's surveillance for antibiotic resistance of *S*. Typhimurium and *S*. Dublin, undertaken first in 1976, is mostly for humans (Franklin, 1997). Generally, the prevalence of resistant *S*. Typhimurium isolated from animals has decreased since 1976. All serotypes except *S.*

Typhimurium and *S.* Dublin isolates were susceptible to "modern quinolones, trimethoprim-sulphonamides, neomycin and gentamicin." The low prevalence of resistant *Salmonella* isolates was attributed to the highly restricted use of antimicrobials as an intervention for *Salmonella* infections in animals. Methods other than antimicrobials are used to eliminate *Salmonella* infections in animals in Sweden.

Resistance of *Salmonella spp.* in Russia

Surveillance for antimicrobial resistance in the USSR began in 1979 and continued at least through 1991 (Panin et al., 1997). In the mid-1980s, the susceptibility of 17,134 *Salmonella* isolates was determined. The *Salmonella* serotypes that have been surveilled are *S.* Typhimurium and *S.* Cholerasuis in pigs and *S.* Typhimurium, *S.* Dublin, and *S.* Enteritidis in cattle. Many isolates from pigs and cattle were resistant to chloramphenicol, tetracyclines, and aminoglycosides. The prevalence of resistance of *Salmonella* isolates from piglets was 20% to 48% for chloramphenicol, 31% to 68% for tetracyclines, and 35% to 40% for neomycin, each range being dependent on factors such as geographic region.

Trends in prevalence of resistance to chloramphenicol, tetracyclines, and aminoglycosides have been recorded in Russia since 1979 (Panin et al., 1997). The trends generally show an increasing prevalence of resistance, regardless of the specific microbe and the antibiotic. The sources of specimens and laboratory methods used from year to year should not be assumed to have been consistent. Chloramphenicol: The prevalence of resistance to chloramphenicol by *Salmonella* isolates from swine abruptly increased from 11.3% in 1979 to 74.6% by 1991 (Panin et al., 1997). Similarly, the prevalence of resistance to chloramphenicol by *Salmonella* isolates from calves increased from 30.3% in 1979 to 61.0% in 1991. Tetracyclines: The prevalence of resistance of *Salmonella* isolated from swine was somewhat stable at 55 to 60.0% for tetracyclines between 1979 and 1991, respectively. However, the prevalence of resistance of *Salmonella* isolated from calves has increased from 37.1% to 58.7% for tetracyclines. Aminoglycosides: The prevalence of resistance of *Salmonella* isolated from swine was 49.0% to 55% for aminoglycosides between 1979 and 1991. The prevalence of resistance of *Salmonella* isolated from calves was 45.0% to 48%.

Panin et al. (1997) suggested that reductions in the frequency of resistance between 1991 and 1998 were explained by decreased manufacturing of antimicrobial drugs in Russia, decreased importation of antimicrobials, and thus decreased use of antimicrobials in livestock production.

Antimicrobial Resistance of *Escherichia coli*

Resistance to *Escherichia coli* in Canada

E. coli isolates are becoming increasingly resistant to some antimicrobials that have been recommended to control *E. coli* (Fairbrother, 1999). A detailed epidemiologic study of resistance of *E. coli* was reported from Canada (McEwen et al., 1997). This observational study of Canadian farms was done to determine the statistical associations between antimicrobial usage in pig production and antimicrobial resistance among fecal *E. coli* isolates of finisher pigs. Finisher

pigs were selected as the study population because of the proximity of their ages to age-at-slaughter. It was assumed that enteric bacteria from feeder pigs were the source of contamination of carcasses. Fresh fecal samples were obtained twice from finisher pigs on each farm.

Multivariate logistic regression analysis was used to test the hypothesis that antimicrobial usage was associated with increased risk of resistance to *E. coli*. The dependent variables were the prevalences of resistance to the respective drugs (i.e., ampicillin, carbadox, gentamicin, nitrofurantoin, spectinomycin, sulfasoxazole, and tetracycline) at break-point concentrations.

The prevalences of *E. coli* resistance to ampicillin, carbadox, gentamicin, nitrofurantoin, spectinomycin, sulfasoxizole and tetracycline were 29.0%, 3.5%, 0.6%, 27.0%, 28.0%, 38.0% and 70.0 %, respectively. This resistance was relatively consistent over the two sampling periods, except for nitrofurantoin and tetracycline resistance. One possible explanation is that two farms began using tetracycline and furazolidone in rations of grower pigs immediately prior to collection of the first set of fecal samples.

The results of McEwen's study were as follows:

1. The risk of ampicillin resistance among *E. coli* was significantly increased with the practice of adding any antimicrobials to weanling rations, tetracycline to grower-finisher rations and penicillins to nursing sow rations.
2. Addition of carbadox to weanling rations was the only significant risk factor in the carbadox resistance model.
3. Similarly, the practice of administering individual treatments of gentamicin to piglets was the only significant factor in the gentamicin resistance model.
4. The risk of nitrofurantoin resistance was increased on farms that added any antimicrobials to weanling rations, but no other factors were significant in the nitrofurantoin resistance model.
5. The risk of spectinomycin resistance was associated with addition of any antimicrobial to grower-finisher rations.
6. The risk of sulfasoxizole resistance was associated with the addition of tetracyclines to grower-finisher rations only.
7. In the case of tetracycline resistance, addition of any antimicrobial to weanling pig rations, and addition of tetracycline to grower-finisher rations were associated with significantly increased risk of resistance.
8. Adding antimicrobials to the ration (i.e., ration therapy, ration medication) was associated with some form of resistance in most regression models, and the effect of ration therapy apparently over-shadowed the effects of individual-animal antimicrobial therapy.
9. Ration therapy of one type or another was significant in all models except the gentamicin resistance model; however, gentamicin was the only drug used exclusively for individual-animal antimicrobial therapy in the study population.
10. Tetracycline and ampicillin resistance were significantly more prevalent on farms where tetracycline and penicillin were used for group therapy, when compared to farms that used these drugs for individual-animal therapy only. Seemingly, individual-animal therapies had less impact on resistance than therapy via the ration.

Resistance of *Escherichia coli* in Sweden

Investigations of antimicrobial resistance of *E. coli* were done in Sweden to document trends in resistance. Most of the isolates of *E. coli* originated from herds in which there were pigs with diarrhea (Franklin, 1997). The frequency of resistance of *E. coli* isolates from pigs did not change dramatically in Sweden between 1981 and 1994 (Franklin, 1984; Franklin, 1997). Resistance to streptomycin decreased slightly and the usage of streptomycin decreased during the same time period by 80.0%. Resistance to tetracyclines is still common. The number of isolates resistant to trimethoprim-sulfa was condidered to be unexpectedly high from 1981 through 1982 (9.0%), although trimethoprim-sulfa had been used only for 6 or 7 years in Sweden.

Resistance of *Escherichia coli* in Russia

The prevalence of resistance of more than 17,000 *Escherichia* isolates from calves in Russia in the mid-1980's was 20% to 68% for chloramphenicol, 36% to 87% for tetracyclines, and 29% to 57% for neomycin (Panin et al., 1997). The prevalence of resistance to chloramphenicol by *Escherichia* isolates from swine abruptly increased from 9.4% in 1979 to 62.1% by 1991. Similarly, the prevalence of resistance to chloramphenicol by *Escherichia* isolates from calves increased from 42.2% in 1979 to 65.6% in 1991. The trends show that the prevalence of resistance of *Escherichia* isolates from swine was stable at 60% to 65% for tetracyclines from 1979 through 1991. However, the prevalence of resistance of *Escherichia* isolates from calves increased from 43% to 73.4% for tetracyclines from 1979 through 1991(Panin et al., 1997).

Resistance of *Escherichia coli* in the U.S.

While data about the prevalence of resistance of *Escherichia coli* from the U.S.'s (NARMS) - Enteric Bacteria is just beginning to become available, results of several other studies have been reported. The prevalence of resistance of *Escherichia coli* to trimethoprim-sulfamethoxazole was 39% for isolates from swine, 46% for isolates from cattle, and 42% for isolates from both (Hariharan et al., 1989; National Research Council, 1998). The prevalence of resistance of these same trimethoprim-sulfamethoxazole resistant, *Escherichia coli* isolates to tetracycline, neomycin, ampicillin, and nitrofurans was 98%, 80%, 74% and 30%, respectively. The prevalence of resistance of *Escherichia coli* from calves with enteritis was 3% to 95% for 10 different antimicrobial drugs in one study (Fairbrother et al., 1978; National Research Council , 1998). In a later study, the prevalence of resistance of *Escherichia coli* from calves with enteritis was 0% to 94% for the same 10 antimicrobial drugs (Coates et al., 1980; National Research Council , 1998).

Antimicrobial Resistance of Other Pathogens
(i.e., pathogens other than those associated with salmonellosis and colibacillosis)

Mastitis

Sweden has done surveillance on antimicrobial resistance of disease agents other than those of salmonellosis and colibaccilosis. *Staphylococcus aureus* has surpassed *Streptococcus agalactiae* as the major cause of bovine contagious mastitis (Stewart, 1999). The cause-specific morbidity for mastitis due to *S. aureus* was 25% in Sweden (Franklin, 1997). About 5% to10% of older and recent *S. aureus* isolates in Sweden are resistant to penicillin, due to their ability to produce penicillinase (Franklin, 1997). Methicillin-resistant *S. aureus* isolates have not been detected in dairy cows in Sweden. The low prevalence of penicillinase-producing isolates in Sweden may be explained by the therapeutic regimen for mastitis. Dry-cow therapy with antibiotics is used only in cows with a history of clinical mastitis during lactation. The antibiotic therapy is aimed directly at the causative bacterial species, and penicillin is never given to cows that are infected with penicillinase producing staphylococci.

Swine Dysentery

Swine dysentery, one of several bloody scours of swine, is caused by the spirochaete *Serpulina hyodysenteriae* (Glock, 1999). Parenteral therapy for swine dysentery may be followed by in-feed therapy or in-water therapy. Because swine dysentery may persist in affected herds, continuous, or at least repetitive therapy, is used to manage the disease. The antimicrobials used in the U.S. are bacitracin, carbadox, gentamicin, lincomycin, sodium arsanilate, tiamulin, tylosin, and virginiamycin. Dimetridazole and ipronidazole are prohibited as therapy against swine dysentery in the U.S. In Sweden, the MICs for *Serpulina hyodysenteriae* were lowest for carbadox, tiamulin, and Ipronidazole, and were highly variable for tylosin (Franklin, 1997).

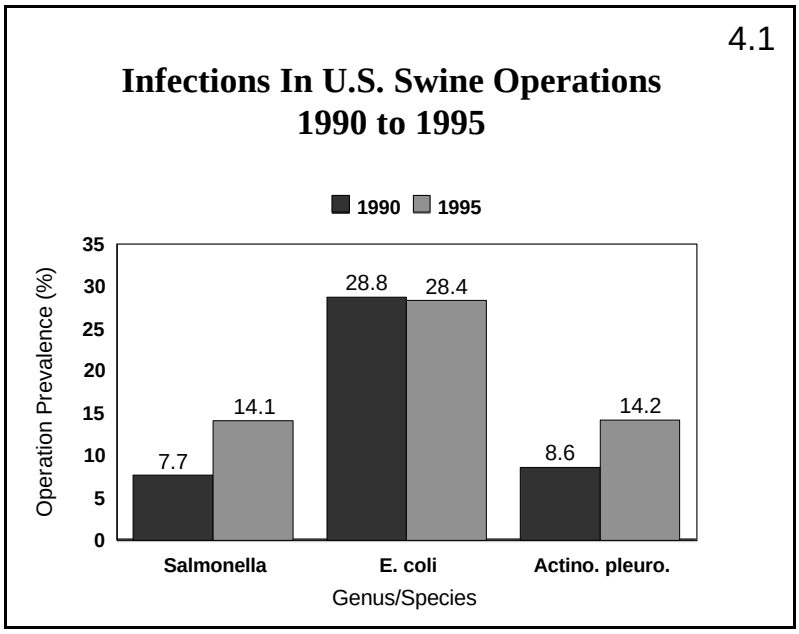
Campylobacteriosis

Campylobacter jejuni and *Campylobacter coli* are associated with diarrheal disease in cattle, sheep, goats and humans (Marshall, 1999). In Sweden, the prevalence of fluoroquinolone-resistant *Campylobacter jejuni* from chickens was less than 5.0%, while about 30.0% of human isolates were fluoroquinolone-resistant (Berndtsson et al., 1996; Sjogren et al., 1993). Some investigators have concluded that chickens may not be the primary source of fluoroquinolone-resistant, *Campylobacter* infections in humans in Sweden because the fluoroquinolone usage in chickens in Sweden was low (Franklin, 1997). All *Campylobacter* isolates from chickens were sensitive to erythromycin.

Respiratory Pathogens

Pneumonia in calves and pigs is associated with *Pasteurella haemolytica*, *Pasteurella multocida*, *Actinobacillus pleuropneumoniae*, and *Haemophilus somnus*. The operation prevalence of diseases that are related to *Actinobacillus pleuropneumonia*e, as reported by swine producers in the U.S., nearly doubled between 1990 and 1995 **(Figure 4.1)**. In Sweden, these pathogens are sensitive to the beta-lactam antibiotics (e.g., penicillin, ampicillin), which are the premier antimicrobials for respiratory infections in pigs, calves, and sheep (Franklin, 1997). Beta-lactamase producing respiratory pathogens have not been isolated from production animals in Sweden (Franklin, unpublished material), and resistance of respiratory pathogens to other

antibiotics is rare in Sweden. In other countries, the prevalence of resistance to penicillin is higher



because of beta-lactamase production (Franklin, 1997).

Novel Antimicrobial-Resistant Pathogens

Salmonella Typhimurium DT104

The primary reservoir of *S.* Typhimurium DT104 is cattle, but the infection has been diagnosed in sheep, goats, pigs, horses, chickens and turkeys (Wall, 1997). One plasmid profile type, characterized by a single plasmid of approximately 60 megadaltons, accounts for most of the increase in both animal and human reports of *S.* Typhimurium DT104 of R-type ACSSuT (ampicillin, chloramphenicol, streptomycin, sulphonamides, and tetracyclines) (Threlfall et al., 1994). An increasing incidence of a multidrug resistant strain of *S.* Typhimurium DT104 in humans was reported in the UK (Threlfall et al., 1994). In 1996 more than 95.0% of *S.* Typhimurium DT104 isolates from humans received by the Public Health Laboratory Service (PHLS) Laboratory of Enteric Pathogens were R-type ACSSuT (CDR, 1997). Multidrug resistant *S.* Typhimurium DT104, with R-type ACSSuT, is now the second most prevalent *Salmonella*, after *Salmonella* Enteritidis PT4, in humans in England and Wales. Isolates of DT 104 that were referred to the PHLS Laboratory of Enteric Pathogens increased from 259 in 1990 to 2,873 in 1994, to 3,837 in 1995, and to 4,006 in 1996 (CDR, 1997).

S. Typhimurium DT104 with R-type ACSSuT is also present in animals and humans in the U.S. For those National Antimicrobial Resistance Monitoring System - Enteric Bacteria (NARMS-EB) isolates from animals, 14 of 137 (10.2%), 45 of 429 (10.5%), and 58 of 328 (17.7%) *S.* Typhimurium isolates were R-type ACSSuT in 1995, 1996 and 1997, respectively (Akkina et al., 1999). Of these *S.* Typhimurium R-type ACSSuT isolates, the proportion that were also phagetype DT104 was 64% in 1995, 22.2% in 1996 and 63.8% in 1997. In the NARMS-EB

study of human isolates, 103 of 306 (34%) and 112 of 321 (34.9%) *S.* Typhimurium isolates were R-type ACSSuT in 1996 and 1997, respectively (Glynn et al., 1998). For the 1996 human isolates, thirteen of the 103 R-type ACSSuT isolates were phagetyped and 85% were DT104, or they were part of the DT104 complex (i.e., other closely related definitive types or closely related untypeable isolates).

The emergence of human and animal isolates of DT104 with decreased susceptibility to ciprofloxacin was preceded by the licensing in the UK in November 1993 of enrofloxacin, a fluoroquinolone used for therapy in all species and for prophylaxis in poultry, calves and pigs (Wall, 1997). Since 1994, isolates with decreased susceptibility to ciprofloxacin (and trimethoprim) have appeared. In 1995, 6.0% of isolates were resistant to ciprofloxacin at MIC:0.25-0.5mg/L, and 27.0% of isolates were resistant to trimethoprim at MIC: >16mg/L (Threlfall et al., 1996). The incidence of ciprofloxacin resistance increased farther in 1996.

During 1994 and 1995, a case-control study was undertaken to investigate risk factors for DT104 infections in cattle in the UK. A case was defined as a farm with one or more symptomatic cattle infected with multidrug resistant *S.* Typhimurium DT104, and a control was defined as a farm selected at random on which no *S.* Typhimurium DT104 infected cattle had been identified. The risk factors identified were: (1) introduction of new stock to the herd, (2) no isolation facilities, (3) stress of calving, and (4) spread of infection by birds and feral cats (Evans, 1996). There was an increased risk of disease when cattle were housed, possibly indicating that persistently contaminated buildings may be a source of infection. Purchasing cattle from dealers, where there is more mixing of stock and thus a greater opportunity for spread of infection, was a greater risk than purchasing cattle directly from other farms (Evans, 1996). Widespread contamination of the environment, equipment, and vehicles was common in the early stages of herd infection. Human cases are often found on farms with infected animals (Wall et al., 1995). The strain has also been identified in domestic pets. Chronic carriage of DT104 for more than 14 weeks has been described in cats (Wall et al., 1995; Wall et al., 1996).

Vancomycin-resistant enterococci

Vancomycin-resistant enterococci (VRE), also referred to as glycopeptide-resistant enterococci (GRE), were isolated from humans in Europe in 1986 and in the United States in 1987 (Goossens, 1997). VRE/GRE have become an important nosocomial infection, especially in the U.S. Glycopeptide-resistance among isolates from farm and pet animals has been found in Europe. The prevalence of *Enterococcus faecium* isolates with vanA-mediated glycopeptide resistance was 8.0% in horses, 8.0% in dogs, 7.0% in chickens, and 6.0% in pigs in Europe. There are few reports of GRE in animals in the U.S., and the reports that do exist involve a few host species only. Specifically, VRE/GRE were not detected in fecal samples of chickens and turkeys in Texas (Coque et al., 1996). Nevertheless, the reports that do exist suggest that VRE/GRE are rare in animals in the U.S. The variations in prevalence between Europe and the U.S. may be due to differences in research methodology, including the type of specimen and laboratory procedures (e.g., failure to use enrichment media). For example, a higher prevalence of carriers of vanA isolates in the intestinal tract was found when stools, rather than swabs, were cultured using enrichment procedures (Jordens et al., 1994).

The results of the Danish antimicrobial resistance monitoring program (DANMAP) have shown that VRE/GRE could be detected among *E. faecium* isolated from Danish pigs, broilers, and cattle from October 1995 to September 1996 (Wegener, 1997). The prevalence of resistance was 29.0%, 59.0%, and 0.0%, respectively, for each species. The preliminary analysis of data for 1997 did not show marked changes in prevalence from previous years.

E. faecium, of the vanA genotype, is the predominant VRE/GRE in livestock and domestic pets in Europe (Goossens, 1997; DeVriese et al., 1996). VanA genotypes have become normal flora in pigs and poultry. Thus, it is possible that organisms with vanA resistance genes may be introduced into humans via the food chain. These colonized humans may introduce the vanA genotypes to human hospitals. The transmission of vanA resistance in this pattern could explain the very high genetic variability of the VRE/GRE isolated from hospitalized human patients in Europe. There are no data to suggest that the situation is similar in the U.S. (Goossens, 1997).

Resistance of enterococci and avoparcin use

There are increasing concerns about the association between resistance of enterococci in animals and food to vancomycin and using avoparcin as a growth-promoter in animal feeds (Bager et al., 1997; Klare et al., 1995b; Aarestrup, 1995; Bates, 1994). Antimicrobials of the glycopeptide class have been used as therapy against infections with multiple, antimicrobial-resistant, Gram positive bacteria in hospitalized human patients (vancomycin) and as an animal feed additive to increase growth rate (avoparcin). Avoparcin has not been available in the U.S. and Canada, but it has been available in many other countries since 1975 (Witte et al., 1997). Avoparcin is used as a growth-promoter for broiler chickens, turkeys, pigs, beef and dairy cattle, calves, sheep, and goats in countries where it has been approved. It was estimated that 24.0 kg of vancomycin was used for human therapy in Denmark in 1994, and 24,000 kg of avoparcin (active compound) was used as feed additives for growth promotion in pig- and broiler production (Wegener, 1997).

The first indication that animals are a reservoir for VRE/GRE came from an analysis in Great Britain (Jordens et al., 1994; Bates et al., 1994). After the emergence of clinical VRE/GRE isolates in a human hospital in Oxford, and the detection of VRE/GRE in fecal samples of both hospitalized and non-hospitalized human patients (Jordens et al., 1994), an investigation of VRE/GRE from humans, farm animals and sewage samples was undertaken (Bates et al., 1994). The *E. faecium* isolates were ribotyped, and 14 distinguishable patterns were found. The different ribotypes suggested that the human hospital was an unlikely origin of the porcine VRE/GRE. In a separate investigation, glycopeptide-resistant *E. faecium* was found in manure from a pig farm and a broiler farm in Germany that fed avoparcin, but not in manure from a poultry farm that did not feed avoparcin (Klare et al., 1995a; Klare et al., 1995b). VRE/GRE were isolated from slurry of another pig farm that fed avoparcin. Cross-resistance to vancomycin, teicoplanin, and avoparcin was found, regardless of the ecological origin of the isolates (Klare et al., 1995a). VRE/GRE were not detected on 17 farms at locations in Germany that did not feed avoparcin (Klare et al., 1995b).

The results of a large study in Denmark in 1995 were similar to those in Germany, i.e., VRE were found in poultry fecal samples from six of eight conventional farms that fed avoparcin, but they were not found in poultry from six farms which did not use feed additives (Aarestrup, 1995). VRE were found not only in poultry and pigs, but also in horses, dogs, and cats during an investigation in Belgium (in 1995), even though avoparcin has not been approved for use in companion animals (DeVriese et al., 1996). This finding raises concerns about the inter-species transfer of VRE/GRE. VRE have been found in large quantities in the liquid medium from thawed poultry and turkey broilers (Aarestrup, 1995; Chadwick et al., 1996). VRE were found in lower quantities in samples of raw minced meat (Aarestrup, 1995).

The EU Commission in 1996 banned avoparcin as a growth promoter as of April 1,1997 (Wegener, 1997c). Recognizing the lack of information about the relationship between resistance to enterococci and avoparcin use, the Commission chose to take a precautionary approach. The U.S. FDA prohibited the extralabel use of glycopeptides in food-producing animals in the United States as of 1997. The FDA's ruling was based on concerns that glycopeptides in food-producing animals would lead to increased risk of transfer of resistant organisms to humans and compromise human therapy (Anonymous, 1997b).

Resistance to streptogramins and virginiamycin

The antimicrobials of the streptogramin family are naturally occurring compounds that are isolated from *Streptomyces pristinaspiralis* (Barri et al., 1992). The streptogramin family is divided into groups A and B, and includes antimicrobials such as the mikamycins, the pristinamycins, the oestreomycins, and the virginiamycins (Le Goffic et al., 1985). Oral pristinamycin (Pyostacine) has been used in Europe for many years to manage staphylococcal infections (Barri et al., 1992).

Virginiamycin is approved by the FDA in the United States for use in chickens, turkeys, swine, and feedlot cattle (Zervos , 1997). Indications for use in chickens includes weight gain, prevention of necrotic enteritis caused by *Clostridium perfringens* and prevention of coccidiosis; in turkeys for weight gain and prevention of coccidiosis; in swine for weight gain and treatment and control of swine dysentery; and in cattle for weight gain and to decrease the incidence of liver abscesses. Virginiamycin is a combination therapeutic that is derived from virginiamycin M (streptogramin A-type) and virginiamycin S (streptogramin B-type) antibiotics.

Quinupristin/dalfopristin is a new streptogramin that has recently (i.e., 1997) completed phase III clinical trials in Europe and in the United States (Zervos, 1997). Quinupristin/dalfopristin is a combination therapeutic that is derived from pristinamycin IA and IIA, respectively. Quinupristin/dalfopristin is expected to be highly efficacious against serious VRE infections of humans.

The prevalence of resistance of *E. faecium* to quinupristin/dalfopristin was as high as 100% in isolates from turkeys in 3 large flocks in Michigan (Zervos, 1997). Quinupristin/dalfopristin-resistant and gentamicin-resistant isolates from turkeys in different culture groups were typed molecularly using pulsed field gel electrophoresis (PFGE) to search for

identical clones. The identical nature of the clones suggested that the isolates had spread among turkeys in the flocks (Donabedian et al., 1995). Higher prevalences of resistance to quinupristin/dalfopristin, ampicillin, and high-levels of gentamicin was found in older turkeys, which may be related to the longer exposure of older turkeys to antibiotics and to animals that are carriers of resistant isolates.

This study did not establish a link between resistant isolates in animals and in humans (Zervos, 1997). Nevertheless, because there is concern about a link between antimicrobials in animal feed and resistant isolates in humans, caution about the use of streptogramins in animals was encouraged by these investigators.

Fluoroquinolone-resistance of *Salmonella spp.* from cattle

France: Quinolones are synthetic antimicrobial agents that are used as therapy in humans and animals against *E. coli* and *Salmonella* infections. The four quinolones that have become available commercially to the French veterinary market are nalidixic acid, oxolinic acid, flumequine, and enrofloxacin (Brisabois et al., 1997). Enrofloxacin, a new generation fluoroquinolone, was approved in France for use in the bovine species in December 1991. Resistance to nalidixic acid, flumequine, oxolonic acid, and enrofloxacin was evaluated using isolates from bovine pathology specimens that were collected in 1995. Most of the 192 isolates of *Salmonella* that were evaluated were of the *S.* Typhimurium serotype. The prevalence of resistance to nalidixic acid was 13%, 6% to flumequine, and 9% to oxolonic acid. None of the isolates were resistant to enrofloxacin. Russia: The resistance of enterobacteria to fluoroquinolones was very low in Russia between 1993 and 1996. After 1996, the use of fluoroquinolones increased. Increased resistance of enterobacteria to fluoroquinolones has been found to be concurrent with increased use of fluoroquinolones (Panin et al., 1997).

Quinolone resistance of *Campylobacter* from poultry

The Netherlands: The predominant reservoir of *C. jejuni* and *C. coli* is thought to be poultry (de Mol, 1994), and *Campylobacters* are food-borne pathogens of humans. Flumequine has been used in veterinary medicine in The Netherlands since the early 1980s. Enrofloxacin was used first in veterinary clinical medicine in 1987, and ciprofloxacin was first used in 1988 (Jacob-Reitsma et al., 1994b). Enrofloxacin is used in broiler production to reduce vaccination problems and to combat respiratory problems due to *Escherichia coli* (Jacob-Reitsma et al., 1994b). No resistant isolates of *Campylobacter* veterinary isolates had been reported in The Netherlands between 1982 and the early 1990s (Endtz, 1991). By 1993 the prevalence of resistance of *Campylobacter* veterinary isolates to quinolones and fluoroquinolones (i.e., nalidixic acid, flumequine, enrofloxacin and ciprofloxacin) was 29.0% (181 of 617 isolates) (Piddock, 1997). Thus, in 1991 Endtz et al. proposed that extensive use of fluoroquinolones in veterinary medicine in meat, poultry and milk production in The Netherlands contributed to the high frequency of fluoroquinolone-resistant *Campylobacters* isolated from humans (Endtz, 1991). Proof of transfer of antibiotic-resistant bacteria to humans via the complicated chain of events involved in poultry farming and food production was difficult. To determine whether broilers that were exposed to fluoroquinolones would provide an environment that would select for fluoroquinolone-resistant

Campylobacters, *Campylobacter*-colonized broilers were exposed to fluoroquinolones (Jacobs-Reitsma, 1994a). When the birds were slaughtered, fluoroquinolone-resistant *Campylobacters* were isolated from all colonized broilers that had been exposed to enrofloxacin. A reassessment of the use of fluoroquinolones in animal husbandry was recommended.

Sweden: The prevalence of resistance to enrofloxacin of 200 *C. jejuni* among 809 *Campylobacter* isolates that were from 6,297 slaughtered chickens in Sweden in 1992 and 1993 was only 4.5% (9 of 200 isolates). None of these flocks had undergone therapy with an antibiotic (Berndtsson et al., 1996). Cross resistance to other quinolones was not observed. The low prevalence of anticrobial resistance in chickens was attributed to the restricted use of these quinolones in poultry production in Sweden.

Spain**:** It has been suggested that, since enrofloxacin was licensed for use in veterinary medicine in Spain in 1990, the increased use of enrofloxacin, flumequine and other quinolones has directly influenced the number of nalidixic acid-resistant *Campylobacters* (Velaquez et al., 1995). The prevalence of resistance has been correlated with dietary concentrations of quinolones, i.e., the prevalence of nalidixic acid-resistant *Campylobacters* increases as the concentrations of quinolones in poultry diets approaches the concentrations of quinolones that are used in the laboratory to select resistant isolates of *Campylobacters* (Velaquez et al., 1995).

United Kingdom: Enrofloxacin was not approved for veterinary use in the United Kingdom (UK) until November 1993, and "little" was used prior to January 1994. Thus, the UK has been referred to as a "control" country to assess the effects of veterinary use of fluoroquinolones on the emergence of fluoroquinolone-resistant, foodborne pathogens (Piddock, 1997). However, it is necessary to distinguish between poultry of UK- and non-UK origins, because much of the poultry consumed in the UK is imported from Europe. A study was done in the UK in 1993-1994, prior to licensing of enrofloxacin there, to assess the effect of veterinary use of fluoroquinolones on the emergence of fluoroquinolone-resistant *Campylobacters* (Gaunt et al., 1996). To do this study, 64 chickens of UK-origin and 50 chickens of non-UK origin were purchased from local supermarkets. The prevalence of *Campylobacter* in the chickens of UK-origin was 57.8% (37 of 64), and only one (2.7 %) of the 37 isolates was resistant to ciprofloxacin. The prevalence of *Campylobacter* in the chickens of non-UK origin was 52.0% (26 of 50), and seven (27.0 %) of the 26 isolates were resistant to ciprofloxacin. Whether there has been an increase in the numbers of ciprofloxacin-resistant *Campylobacters* isolated from UK-bred chickens, now that enrofloxacin has been approved, has not been shown yet; however, the numbers of resistant *Campylobacter*s in the one localized area in the UK is increasing.

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